Vegetative Propagation of *Aquilaria cumingiana* (Decne) Ridl.: Effects of IBA Concentration and Leaf Trimming

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Abstract

Aquilaria cumingiana (Decne) Ridl. is considered among the agarwood-producing species (APS) that grows naturally in the Philippines. With the government's restriction in trade and collection, APS domestication using vegetative propagation to address the problem of seed supply for mass production of seedlings for plantation establishment has been found the best option. This study assessed the effect of various indole-3-butyric acid (IBA) concentrations (0, 250 and 500 ppm), different leaf trimmings (trimmed 0, 25 and 50% of the leaf surface area) and their interaction using young and juvenile leafy stem cuttings. Parameters evaluated include percent rooting (PR), number of roots (NR), number of secondary roots (NSR), length of the longest roots (LLR), and average root length (ARL). While non-significant effects were obtained from most of the root traits evaluated, results of the analysis of variance (p > 0.05) detected a significant effect in the case of LLR treated with 500 ppm of IBA (15.25 mm, p = 0.039) and NSR in cuttings with untrimmed leaf laminas (5.19, p = 0.028). A significant effect was also generated from IBA and leaf trimming interaction in terms of the NSR, particularly between untrimmed cuttings (OW) and 250 ppm IBA (6.48, p = 0.049). Generally, the results of the study reflect the ability to mass produce the A. cumingiana using unsophisticated techniques of macro-somatic cloning.

***Keywords**: Aquilaria cumingiana*, IBA, leaf trimming, agarwood

1. Introduction

Agarwood is an expensive fragrant resinous forest resource that is produced as a response to various pathological processes of either complex biotic (pathogen or fungal infection) and abiotic (natural injuries and chemical application among others) stressors (Suharti, 2011; Tan *et al.*, 2019). Interestingly, APS with the most damaged, infected, and diseased woods are far more desirable than healthier ones (Naziz *et al.*, 2019). It is derived from Thymelaeaceae family, particularly of the genus *Aquilaria* (Lee and Mohamed, 2016). Currently, it is the world's costliest timber-based product that is far more expensive than gold, which is commonly referred to as "liquid gold" (Xu *et al.*, 2013; Naziz *et al.*, 2019).

APS under the genus *Aquilaria* occurs mainly in South and South-East Asia (Persoon and van Beek, 2008). From 22 accepted scientific names (The Plant List, 2013), 13 are currently recognized as scented resin producers (Lee and Mohamed, 2016). Nine are found in the Philippines with six identified as endemic (Lee and Mohamed, 2016), namely *Aquilaria apiculata* Merr., *A. brachyantha* (Merr.) Hallier f., *A. citrinicarpa* (Elmer) Hallier f., *A. parvifolia* (Quisumb.) Ding Hou, *A. urdanetensis* (Elmer) Hallier f., *A. decemcostata* Hallier f., *A. filaria* (Oken) Merr., *A. malaccensis* Lam. and *A. cumingiana* (Decne.) Ridl. The last three *Aquilarias* have been recognized to produce the agarwood (Convention on International Trade in Endangered Species of Wild Fauna and Flora [CITES], 2004; Adelina *et al.*, 2004; Lee and Mohamed, 2016; Lee *et al.*, 2018b; Tan *et al.*, 2019).

Pioneering work on APS in the Philippines focused mainly on general characterization of tribe *Aquilarieae* (Quisumbing, 1946). While basic studies on biology, botany, ecology, geographic distribution and medicinal uses of *A. cumingiana*, locally known as "Palisan" (Tagalog), "Bago" (Manobo) and "Binukat" (Aklan Bisaya), have been documented; most publications talk primarily on its conservation and biodiversity status (CITES, 2004; Langenberger *et al.*, 2006; Japan International Cooperation Agency, 2011; Lennertz and Scahde, 2017; Department of Environment and Natural Resources [DENR], 2017; Plant Resources of South-East Asia, 2019). In fact, despite formally recognized as among APS, little did mention about strategy to mass produce this species (CITES, 2004; Lee *et al.*, 2018b). Numerous studies, however, have been reported on the other *Aquilarias*, like in the case of *A. malaccensis* and *A. crassna* (Soehartono and Newton, 2001; CITES, 2003, 2004; International Tropical Timber Organization, 2011; Sitepu *et al.*, 2011; Saikia and Khan, 2012; Lee *et al.*, 2013; Chhipa and Kaushik, 2017; Sen *et al.*, 2017; Sampson, 2017; Lee *et al.*, 2018a; Nasution *et al.*, 2019).

The increasing demand for agarwood as exacerbated by sky-rocketing prices in the global market expedites the decrease of *Aquilaria* populations in the wild. Rampant harvesting and hacking – deliberately wounding the trees – of even the young and living uninfected trees are prevalent (Persoon and van Beek, 2008; Sitepu *et al.*, 2011; Lee and Mohamed, 2016; Esyanti *et al.*, 2019). Not only these practices are accelerating the decline of their naturally growing population, but it also threatens their very existence by affecting their reproduction cycle (Lee and Mohamed, 2016). To abate this problem, other countries have enacted laws restricting the species trade and collection. In Indonesia, both the genus *Aquilaria* and *Gyrinops* have been included in CITES Appendix II, while substantial regulatory measures in chain-of-custody have been implemented in Malaysia (CITES, 2004; Lee and Mohamed, 2016; Lian *et al*., 2016). In the Philippines, the DENR has implemented trade and collection restrictions both locally and abroad (Protected Areas and Wildlife Bureau, 2003). Eight species of *Aquilaria* are also included in the list of Philippine Threatened Plant Species (DENR, 2017).

However, many still believe that plantation establishment rather than pure collection and trade restriction is still the best option to satisfy the increasing demand while reducing the pressure on wild populations (Sitepu, 2011; Yin *et al*., 2016; Turjaman and Hidayat, 2017). *Aquilaria* can produce thousands of seeds per tree annually (Soehartono and Newton, 2001). However, rampant harvesting in the wild hampered the seedling production (Lian *et al*., 2016; Lee and Mohamed, 2016). Additionally, the seed is recalcitrant with low viability and percent germination; hence, storage is not feasible (Kundu and Kachari, 2000; Sitepu, 2011; Tabin and Shrivastava, 2014). To address the problem, some applied micropropagation as an alternative for seedling production (Chiu, 2016; Esyanti *et al*., 2019). Others tried macropropagation using young (Loc and Luu, 2002; Yung, 2013; Yusnita *et al*., 2017) and matured stem cuttings (Borpuzari and Kachari, 2018). Unfortunately, aside from sophistication, most of such strategies were done heavily on a few *Aquilaria*, particularly *A. malaccensis*, but nothing or only limited on other species like *A. cumingiana*, particularly in the Philippines. Therefore, this study was conducted to determine the ability to mass produce the species vegetatively using a low-cost rooting chamber. Specifically, it tested the effect of various IBA concentrations, different leaf trimmings and their interaction in rooting of young and juvenile cuttings.

2. Methodology

2.1 Plant Material

Seeds of *A. cumingiana* were collected from identified mother trees in the Rajah Sikatuna Protected Landscape (RSPL) and the Forest Academic

Research Area of Bohol Island State University (BISU) in the island province of Bohol, Philippines in October 2019. Confirmation of the locations of those mother trees was based on the plant database in BISU and the published articles of Reyes *et al*. (2015) and Aureo *et al*. (2020). Collected seeds were processed, germinated and transplanted in polyethylene bag (6" x 8") with formulated potting medium composed of topsoil and organic compost (1:1 ratio of garden soil and carbonized rice hull) using the makeshift forest nursery facilities of BISU. From some 500 seedlings produced, about 200 were transported via commercial airplane from BISU to Forest and Agroforestry Nursery Learning Laboratory (FANLL) of the Institute of Renewable Natural Resources in the College of Forestry and Natural Resources at University of the Philippines Los Baños, Laguna (IRNR, CFNR, UPLB).

Following the procedure applied by Piñon *et al.* (2012), bare-rooted seedlings were packed in a Styrofoam box, added with ice and covered with banana sheath before sealing the lid with packaging tape and label. Young shoots (about 3.81 cm length) with three to five leaves were harvested and leaf trimmed. Three types of trimmings were used. One-half (OH) (about 1.99 cm^2) had 50% of the leaf surface trimmed, three-fourth (TF) (about 3.13 cm^2) had 25% of the leaf surface trimmed and one whole (OW) (about 5.21 cm^2) had 0% of the leaf surface trimmed. Cuttings were then sanitized using fungicide (Dithane M-45, Dow Agrosciences, United States) solution after dissolving 1 tablespoon of fungicide powder in 8 L of water. After that, cuttings were air-dried for about 5-7 min before dipping in various IBA concentrations for about 10 min before planting in the improvised rooting chamber. Cuttings were profusely watered twice daily (7:00-8:00 AM and 5:00-6:00 PM) using a 2.5-L hand-pumped mist sprayer. Both rooted and unrooted cuttings were harvested 50 days after planting.

2.2 Improvised Rooting Chamber

The rooting chamber used in the study (Figure 1a) is based on the low-cost non-mist propagator firstly used by Leakey *et al.* (1990a). It is simpler with less sophistication and relatively more resistant to termites compared with wood-based rooting chamber used for various tropical tree species (Leakey *et al.*, 1990a; Ahmad, 2006; Leakey, 2014). The curvature of the half-drum provides a good drainage system that could minimize fungal infection due to waterlogging. However, aside from daily watering, this chamber may not be applicable for trees with relatively large leaf surface area and long internodes. The 50-100 cm height of the chamber (Leakey *et al.*, 1990a) is also advantageous for air circulation compared with 16-27 cm in the present study. Moreover, erratic temperature fluctuation, especially during summer is more likely due to a more compact interior space. Hence, it is suggested to place this chamber under shaded areas and use it only during rainy season when microclimate condition is relatively more stable.

This chamber is made up of a 200 L blue plastic drum, which was cut in half in a lengthwise direction. Two drainage holes (4 cm^2) were made at the bottom. One line of stones (3-8 cm) was laid with gravels placed and filled up the empty spaces in between the stones. On top of it, a layer of black plastic net (1.5 cm x 1.5 cm) was laid before adding gravel (0.5-1.0 cm) to a total center depth of 12-15 cm. Another two layers of black net were placed on top of the gravel before adding the washed river sand (5-8 cm depth). A welded wire mesh (60 cm x 95 cm) with 2 x 2 inches square opening and 0.12 inch diameter was painted with chocolate brown paint (quick-drying enamel, Boysen, Philippines) before installing on the opening (Figure 1b). Finally, a single piece of clear polyethylene (gauge number 8) was used to cover it and closely fitted with plastic straw rope (Figure 1b).

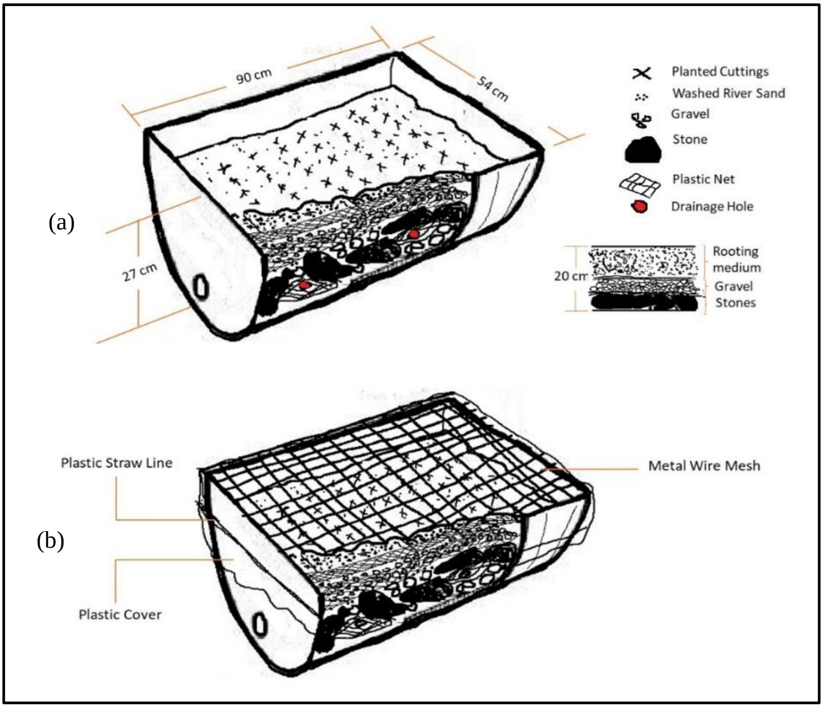


Figure 1. Layout of the cuttings planted in the improvised plastic half-drum rooting chamber covered with a metal frame (a) and transparent polyethylene plastic (b)

2.3 Experimental Design and Data Analysis

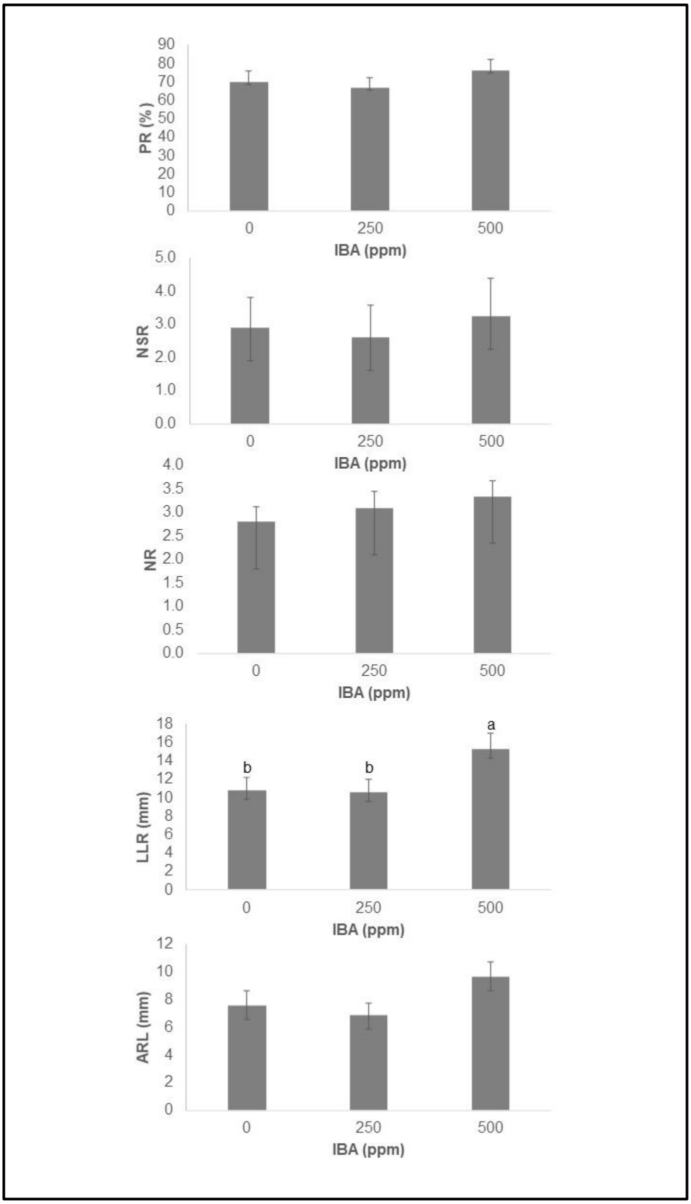
The study used a 3 x 3 factorial in complete randomized design. Treatments were various IBA concentrations (0 ppm [distilled water]; 250 and 500 ppm) and different leaf trimmings (removed 0, 25 and 50% of leaf surface area) with a total of nine possible treatment combinations replicated three times. A total of 189 cuttings were planted with seven cuttings per replication and 21 cuttings per treatment.

Assessment of the treatment effects was done after 50 days using five parameters. These included percent rooting (PR) (total number of cuttings rooted over the total number of cuttings planted multiplied by 100), number of secondary roots (NSR), number of roots (NR) (total number of roots produced per cutting), length of the longest root (LLR) (longest root produced per cutting, measured using a ruler) and average root length (ARL) (average length of produced roots per cutting, measured using a ruler). Data gathered were arranged, organized and transformed [log10 and log(x+1)] for normality distribution using Microsoft Excel Office 365 program before statistical analysis. All statistical computations including analysis of variance (ANOVA) followed by post-hoc test using the Duncan Multiple Range Test were performed using R-Statistics software version 3.6.3 (2020).

3. Results and Discussion

3.1 Effect of IBA

Figure 2 shows high overall mean rooting percentages (68.25-74.60%), despite non-detection of any significant effect ($p > 0.05$) due to IBA treatments except the LLR. Among the concentrations, cuttings treated with 500 ppm had a significant increased ($p = 0.039$) in LLR (15.25 mm) (Figures 2 and 3). These results suggest two important findings. First, high rooting percentages obtained even in control treatment (0 ppm) demonstrates the possible existence of naturally occurring endogenous auxins. Secondly, the significant increase in LLR indicated that higher IBA concentration might potentially enhance the root length of *A. cumingiana*.



ARL – average root length (ARL); LLR – length of the longest roots, NR – number of roots, NSR – number of secondary roots; and PR – percent rooting. Error bars represent the standard error. Means followed by the same letter(s) are not significantly different at 5% level according to Duncan's Multiple Range Test.

Figure 2. Effect of IBA on rooting ability of *A. cumingiana*

Successful rooting even in control treatment (0 ppm) has been recorded in many tree species like *Melicia excelsa*, *Nauclea diderrichii*, and *Chrysophyllum albidium* G. Don (Ofori *et al*., 1996; Leakey *et al.*, 1990b; Boateng, 2013). After studying the physiological effects of IBA in plant growth and development, Ludwid-Muller (2000) explained that despite previously identified as synthetic auxin, mounting evidence proved that IBA occurs naturally in plants. Several researchers support this claim (Cleland, 1999; Srivastava, 2002; Calio *et al*., 2006; Costa *et al.*, 2017; Frick and Strader, 2018). These indicate that certain amount of auxin could be found in *A. cumingiana;* hence, high rooting was recorded even in untreated cuttings. However, since phytochemistry test was not undertaken, the authors of the present study cannot specifically identify the auxin that is present in species studied.

Using lignified stem cuttings, Borpuzari and Kachari (2018) applied IBA with concentrations ranging from 0 to 8000 ppm and discovered that 1000 ppm had significantly increased the length of roots of *A. malaccensis*, particularly against higher concentrations (4000 and 8000 ppm). In contrast, with 0 to 200 ppm IBA applied in *A. microcarpa* (two to eight years old), Yung (2013) found that untreated cuttings generated the optimal performance in all traits evaluated including root length. In the present study, cuttings treated with 500 ppm of IBA had significant increase in LLR. These outcomes imply that while non-application of IBA is feasible to mass produce the *A. cumingina*, low concentrations do not provide any effect and the use of higher concentrations could potentially enhance the LLR as the latter enhances the ethylene production (Ludwid-Muller, 2000).

The positive effect of ethylene in the adventitious root formation has been reported in various plants (Robbins *et al*., 1985; Wang and Pan, 2006; Iqbal *et al*., 2017). However, caution must be made not to apply an extremely high concentration (e.g., 4000 to 8000 ppm by Borpuzari and Kachari [2018]) since phytotoxicity is highly likely with negative effect on rooting (Costa *et al.* 2017). Such phytotoxicity is probably the result of change in the relative sink strength at the root initials with an increased amount of ethylene due to IBA-induced basipetal transport of assimilates (Robbins *et al.*, 1985; Hartmann *et al*., 1990). Inhibitory effects of high auxin concentrations have been discovered in *Casuarina sumatrana* (de Vriese) L. Johnson, *Cordia alliodora* (Ruiz & Pavon), and *Pongamia pinnata* (L.) (Goh *et al*., 1995; Mesen *et al*., 1997; Kesari *et al*., 2008).

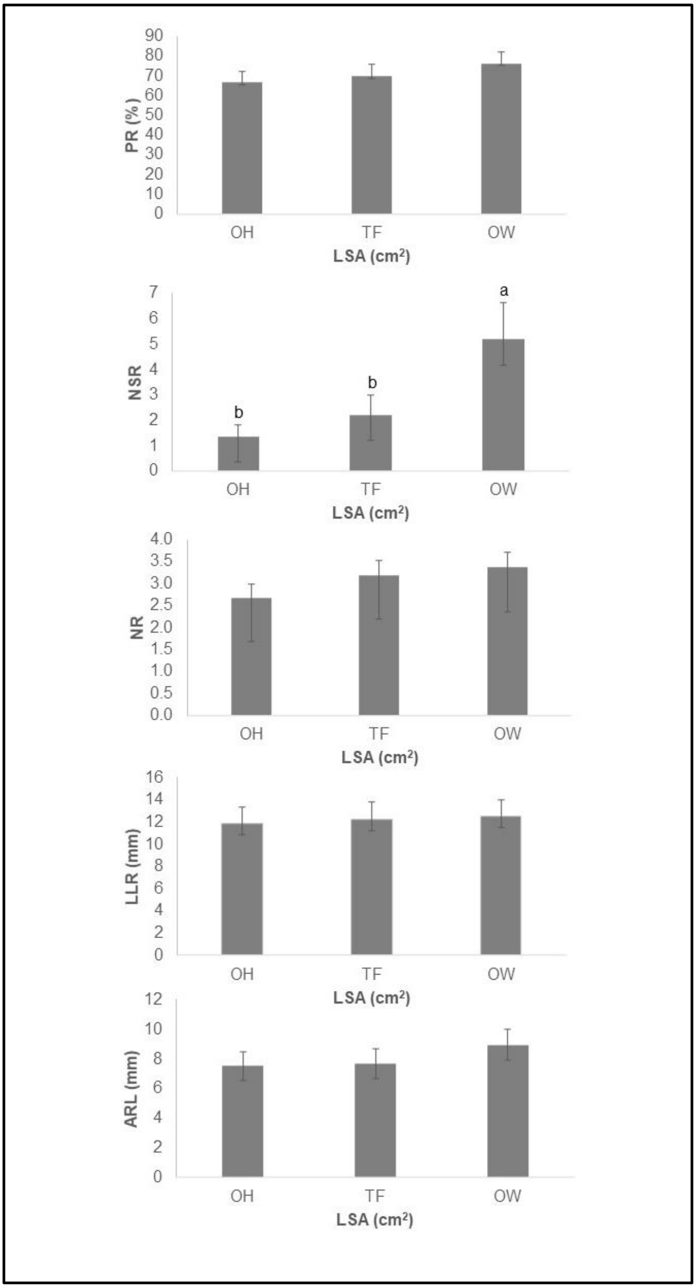
3.2 Effect of Leaf Trimming

Majority of rooting responses due to varying leaf surface areas (LSA) with leaf trimmings did not vary significantly aside from the NSR. The overall mean values in NSR for OH and TF cuttings were 1.37 and 2.22, respectively (Figure 3) while a significant increased ($p = 0.028$) in NSR (OW = 5.19) was detected in untrimmed cuttings (Figures 3 and 4). These imply that variations on leaf laminas did little effect on rooting induction while retaining an intact LSA might have contributed significantly to the production of secondary roots.

With the effect of transpiration, stomatal dysfunction and limited water uptake, newly planted cuttings are most vulnerable to wilting before root initiation (Gates, 1968; Fordham *et al*., 2001; Peter, 2010; Leakey, 2014). As such, in many vegetative propagation studies, leaf trimming is done to prevent this water loss, particularly in trees with large photosynthetically active leaves (Leakey *et al*., 1990b; Longman, 1993; Leakey, 2014).

Previous reports did not mention the effect of varying leaf sizes in rooting induction of *Aquilaria*. They did compare the effect of various synthetic auxins, rooting media, and the response to auxin of leafless cuttings using *A. malaccensis* (Yusnita *et al.*, 2017; Borpuzari and Kachari, 2018), but none on leaf trimming. However, numerous studies have been reported on other tree species with up to 100 cm^2 of the LSA were trimmed. Examples include *Eucalyptus camaldulensis, Khaya ivorensis,* and *Azadirachta indica* (Geary and Harding, 1984; Leakey *et al.*, 1982; Leakey *et al*., 1990b; Tchoundjeu and Leakey, 1996; Kamaluddin and Ali, 1996).

Generally, they agreed that an increase in LSA has a corresponding increase in the rate of rooting. In contrast, result of the present study was inconsistent in *Terminalia spinosa* Engl. (Newton *et al*., 1992) and *Khaya senegalensis* (Ky-Dembele *et al.*, 2011) – the variation in leaf area had little effect on all rooting parameters except on the NSR. Finally, although non-significant, the overall mean percent rooting of untrimmed cuttings (OW = 76.19%) was found relatively higher than those with portion of the leaf surface removed (TF = 69.84% and OH = 66.67%) (Figure 3). This suggests that instead of enhancing, trimming the leaves might adversely affecting the rooting at least on the subject *Aquilaria*.



ARL – average root length (ARL); LLR – length of the longest roots, NR – number of roots, NSR – number of secondary roots; and PR – percent rooting. Error bars represent the standard error. Means followed by the same letter(s) are not significantly different at 5% level according to Duncan's Multiple Range Test.

Figure 3. Effect of leaf trimming on the rooting ability of *A. cumingiana*

Meanwhile, the significant increase in NSR of untrimmed cuttings compared with those trimmed may be explained in three things. Firstly, it is probably due to the ability of the untrimmed cuttings to photosynthesize to produce the assimilates while they are planted in the chamber (Newton *et al*., 1992; Leakey 2004, 2014). Others, however, suggested that the presence of carbohydrate reserves in large-size cuttings might influence the production of secondary roots (Ky-Dembele *et al*., 2011). Since this study did not use large-size cuttings, it was probably more of the former rather than the latter. Secondly, since untrimmed cuttings do not have an open wound, less likely that stomatal dysfunction (Fordham *et al*., 2001) occurs; hence, assimilates production is more effective and efficient. Finally, excessive leaf abscission is normally encountered in large leaf-size tree species, especially if untrimmed (Newton *et al*., 1992; Leakey, 2014), but the same phenomenon did not occur in the present study, even among untrimmed cuttings (about 5.21 cm^2). Perhaps, such leaf area satisfies the requirement on balance between photosynthesis and transpiration (Leakey, 2004); hence, favoring the production of secondary roots. This suggests that leaf trimming might not be necessary for vegetative propagation of *A. cumingiana*.

3.3 IBA and Leaf Trimming Interaction Effect

IBA and leaf trimming Interaction did not vary significantly for most of traits examined except on NSR. It was almost undetected, however, as the value obtained was nearly equal to 5% level of significance (Table 1). Among interactions, the highest NSR was obtained from untrimmed cuttings confirming the effective use and production of assimilates in terms of secondary roots initiation. After comparing the means, interaction between untrimmed cuttings (OW) treated with 250 ppm achieved the highest NSR (6.48) (Table 1 and Figure 4).

Both interactions between OH/IBA and TF/IBA generated significant effects at 5% level of significance in terms of NSR. Among OH/IBA interaction, cuttings with 50% of the LSA trimmed and treated with 500 ppm obtained a significant increase in NSR (3.24). While among the TF/IBA interaction, cuttings with 25% of the LSA removed and treated with 250 ppm had a significant increase in NSR (1.05).

Table 1. Interaction effect of different IBA concentrations and various types of leaf trimming (OH of cuttings *of A. cumingiana,* such as the PR, NR, NSR, LLR, and ARL. OH (trimmed 50% of the leaf surface) TF (trimmed 25% of the leaf surface) and OW (trimmed 0% of the leaf surface).

LSA/IBA	PR (%)	NR	NSR	LLR (mm)	ARL (mm)
OH					
0 ppm	66.67	2.19	0.52[b]	9.64	6.67
250 ppm	57.14	2.10	0.33[b]	7.67	4.83
500 ppm	76.19	3.71	3.24[a]	18.33	11.12
TF					
0 ppm	61.90	3.10	3.48[a]	10.05	6.46
250 ppm	71.43	3.29	1.05[b]	9.64	5.74
500 ppm	76.19	3.19	2.14[a]	17.05	10.85
OW					
0 ppm	80.95	3.10	4.71[a]	12.76	9.65
250 ppm	76.19	3.90	6.48[a]	14.43	10.02
500 ppm	71.43	3.10	4.38[a]	10.38	7.04
SE	1.95	0.19	0.58*	0.88	0.59

*significantly different

Although non-significant, results of the interaction between IBA and LSA with the highest percent rooting was recorded between untreated (0 ppm) cuttings with the leaves remain intact (OW/IBA 0 ppm = 80.95%) (Table 2). This suggests that such interaction was so small to be detected after seven weeks, but a significant effect might be observed once rooted cuttings were transferred into individual containers with nutrient-improved growth media for a longer period. This was the case of *Azadiractha indica*, where IBA and LSA interaction effect on rooting was initially non-significant but not after 12 weeks when rooted cuttings were transferred from non-mist propagator to polythene pots (Kamaluddin and Ali, 1996).

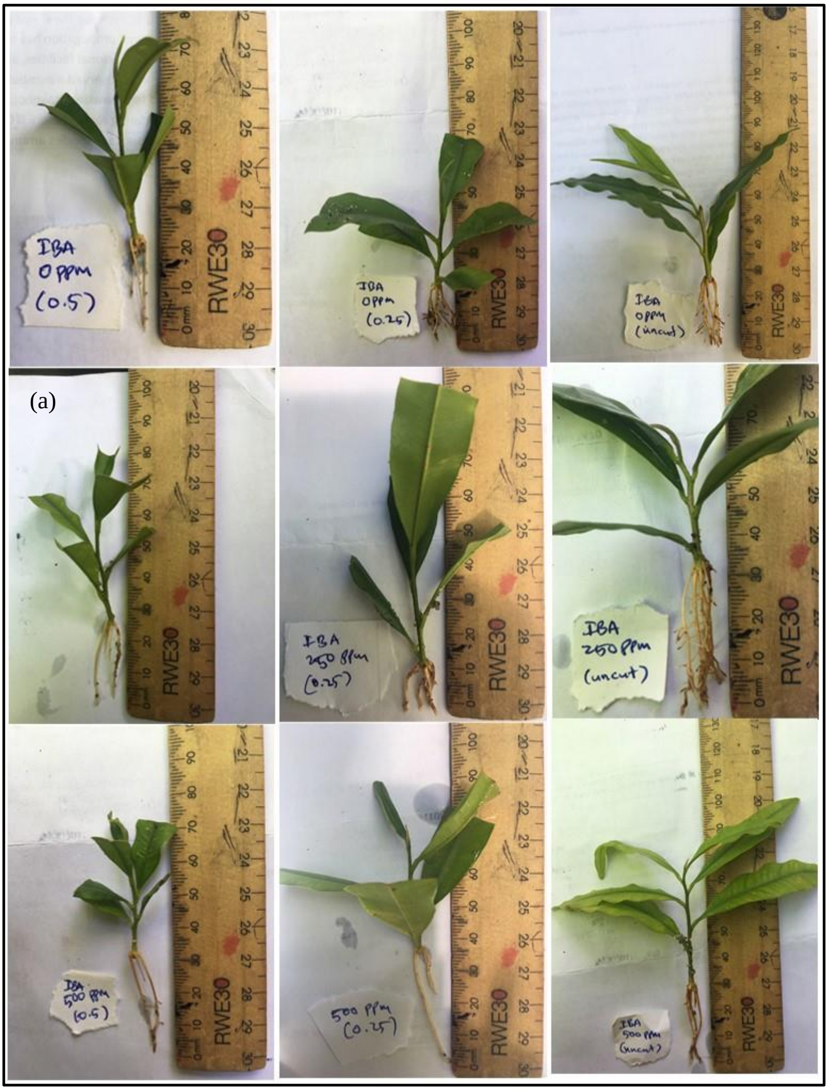


Figure 4. Samples of *A. cumingiana* showing the cuttings with induced roots as treated with various IBA concentrations (0, 250 and 500 ppm) and leaf trimmings (removed 0, 25 and 50% of the leaf surface area). Note the pronounced LLR with cuttings treated with IBA (500 ppm) and the NSR of the untrimmed cuttings. The unlabeled cutting (a) was treated with IBA (250 ppm) and leaf trimmed (50%).

4. Conclusion and Recommendation

No report has been mentioned about the possibility of domesticating the APS, particularly *A. cumingiana* using macro-somatic cloning in the Philippines. In the present study, no significant effects were detected in most of the root traits evaluated for IBA, leaf trimming and their interactions. The non-significant effect of IBA implied the presence of naturally occurring endogenous auxins while those of varying leaf laminas would indicate that leaf trimming had little effect on rooting. The same observation was found when studied their interaction suggesting that vegetative propagation using young and juvenile seedlings of *A. cumingiana* would be feasible even without auxin and leaf trimming application.

Meanwhile, a significant effect of IBA in LLR, particularly those applied with 500 ppm, imply that high auxin concentration enhances the production of ethylene, which improves the length of roots induced. Significant increase in NSR in cuttings with untrimmed leaf laminas compared with those trimmed reflects a more efficient use and production of assimilates as open wound was not created; hence, stomatal dysfunctions are avoided. A significant effect between IBA and LSA interaction on NSR indicates an improved secondary roots production with intact leaf laminas.

5. Acknowledgement

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